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The Circular Dichroism of Phosvitin*

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SUMMARY

Circular dichroism spectra between 185 and 320 $m\mu$ were obtained on phosvitin at pH 3.4 and 6.6. A strong negative band at 198 $m\mu$ dominates both spectra. At higher wave lengths, a negative shoulder at 225 $m\mu$ is present at pH 3.4, although at pH 6.6, two weak bands, one positive, the other negative, are observed at 220 $m\mu$ and 233 $m\mu$, respectively.

In a recent report, Perlmann and Allerton (1) have described the optical rotatory dispersion curves of phosvitin at acid, neutral, and alkaline conditions. This protein contains only 15% of nonpolar amino acids (66% are anionic; 17% are cationic) and thus may be expected to behave similarly to charged polypeptides, such as polyglutamic acid (2). The observation of Perlmann and Allerton that, when pH is shifted from 6.6¹ to 3.4, the character of the optical rotatory dispersion curve shifts from one with a minimum at 205 $m\mu$ to a bimodal one with troughs at 205 and 232 $m\mu$ suggests a transition from a predominantly unordered conformation to one that contains α -helical or β -structured regions. This finding is quite consistent with the observations of Jirgensons (3) that the viscosity and optical rotation of this protein change with pH in a manner typical for a flexible polyelectrolyte. In order to gain a greater insight into the conformation of this protein and its changes with pH, it was examined under similar conditions by the methods of circular dichroism and infrared spectroscopy and the results are reported in this communication.

The phosvitin sample was the same as used by Perlmann and Allerton. The pH 6.6 solutions were prepared as before (1), although the pH 3.4 solutions were obtained by dissolving phosvitin in water and adjusting the pH with acetic acid. The circular dichroism spectra were measured from 320 to 185 $m\mu$ with a Durrum-Jasco ORD/UV5 apparatus,² with the use of cells of 10.0, 1.0, and 0.11 mm in thickness and protein concentrations of 0.19 to 0.70 g per liter, with reasonable overlap between the

various conditions. The infrared spectra were obtained on a Beckman IR7 spectrophotometer² with matched cells of 0.1-mm path length. For the infrared experiments, 16.0 g per liter solutions of the protein were made in D₂O and adjusted to pD values corresponding to the pH values of the circular dichroism measurements. Concentrations were determined by micro-Kjeldahl analysis with the use of a phosvitin nitrogen content value of 13.6%. Theoretical optical rotatory dispersion curves were calculated from the circular dichroism data with the Kronig-Kramers transform (4-7) by using a Fortran program as described previously (8).³

The circular dichroism results at pH 6.6 and 3.4 are presented in Figs. 1 and 2. In neither case was there any absorption in the region between 320 and 250 $m\mu$. At pH 6.6, the spectrum is characterized by a strong negative band at 198 $m\mu$ and two weak bands, one positive at 219 $m\mu$ and one negative at 232 $m\mu$. The spectrum crosses the base-line at 187 $m\mu$, indicating the presence of a positive band at a lower wave length. The shoulder at 194 $m\mu$ is reproducible, but no attempt was made to analyze it as a separate band. The circular dichroism pattern obtained at pH 6.6 is qualitatively similar to spectra observed with polypeptides in unordered conformation (8-11). The presence of positive and negative peaks in the region of 220 and 235 $m\mu$ is typical of such structures; in the case of phosvitin, however, the ratio of the amplitudes of these bands is considerably different from that observed in synthetic polypeptides (10, 11), although the intensity of the strong negative band below 200 $m\mu$ is considerably less than that observed with model compounds (9, 11). Although the apparent negative band at 233 $m\mu$ may not be real,⁴ but only the tail of the strong 198 $m\mu$ band, the spectrum in the present case was resolved into three bands for calculating the corresponding optical rotatory dispersion curve shown in Fig. 1. This was performed by projecting about the maximum of each peak, the side which did not overlap with any band and repeating this process until three gaussian bands were obtained. The calculated optical rotatory dispersion pattern is compared with the experimental curve in the right-hand side of Fig. 1. This calculated curve is dominated by a negative Cotton effect at 198 $m\mu$ having a trough at 205 $m\mu$ and a peak at 190 $m\mu$, with small contributions from the weak "bands" at higher wave lengths.

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¹ The final pH attained when phosvitin is dissolved in pH 7.7 phosphate buffer of 0.1 ionic strength.

² Mention of the above does not imply endorsement by the United States Department of Agriculture over others mentioned.

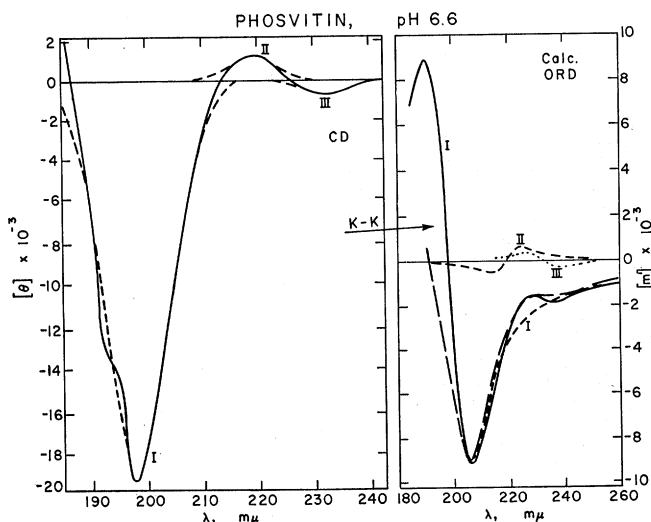


Fig. 1. Circular dichroism (CD) of phosvitin at pH 6.6 (0.1 ionic strength phosphate buffer). Left, the solid line is the experimental curve; the dashed lines represent the bands (I, II, III) into which the pattern was decomposed. Right, calculated optical rotatory dispersion curve (Calc. ORD) with the use of the Kronig-Kramers (K-K) transformation; short dashed and dotted lines represent the individual Cotton effects given by the three bands; solid line is their sum; the long dashed lines represent the experimental curve (1).

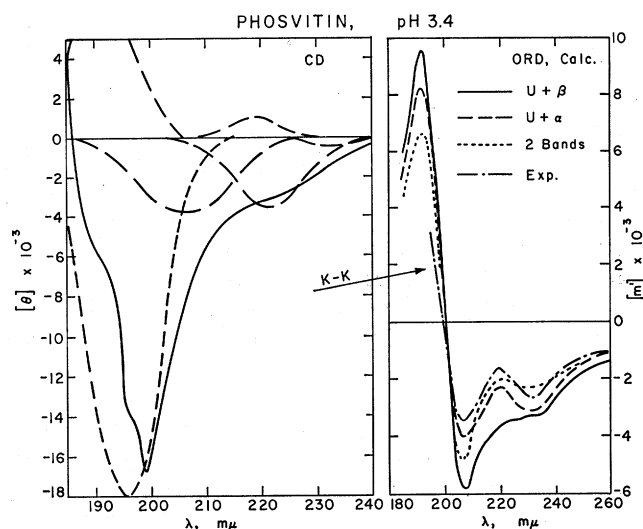


Fig. 2. Circular dichroism (CD) of phosvitin at pH 3.4 (aqueous solution adjusted to pH with acetic acid). Left, the solid line is the experimental curve; the dashed lines represent the bands (three unordered, three α -helical) into which the spectrum was decomposed. Right, calculated optical rotatory dispersion curves (ORD, Calc.) with Kronig-Kramers (K-K) transformation; solid line, unordered and antiparallel β ; dashed line, unordered and α helix; dotted line, two negative bands at 199 and 222 $m\mu$; dot-dashed line, experimental curve (1).

The agreement between the calculated and experimental (1) curves is excellent. Both display a deep trough at 205 $m\mu$ ($[m']_{\text{exp}} = -9.0 \times 10^3$; $[m']_{\text{calc}} = -9.2 \times 10^3$) and a cross-over point at 198 $m\mu$. Above 205 $m\mu$, the rotation becomes rapidly less negative up to 225 $m\mu$, above which wave length the curve flattens and a small trough appears at 236 $m\mu$. Detailed examination of the calculated curve shows that this trough reflects the presence of the weak dichroism above 215 $m\mu$. The calculated curve has another peak at 190 $m\mu$. Comparison of these optical rotatory dispersion curves with data obtained on standard polypeptides shows a striking similarity to curves obtained with poly-L-glutamic acid and poly-L-lysine in the unordered conformation (11-14)⁵; these are characterized by a trough at 204 to 205 $m\mu$, a peak at 190 to 191 $m\mu$, a cross-over point at 197 $m\mu$, and a sharp increase in negative rotation in the region below 225 $m\mu$.

Other structures which display similar optical rotatory dispersion characteristics include collagen (16), with a trough at 207 $m\mu$, a cross-over point at 197 $m\mu$, and a flattening of the wave length dependence of rotation above 220 $m\mu$, and poly-L-proline I which has a trough at 207 $m\mu$ (10, 16-18)⁶; in the last case, however, there is also a peak at 223 $m\mu$ and a cross-over point at 215 $m\mu$. In view of the special structures of collagen and polyproline I, and the small amount of proline (1.5%) in phosvitin (2), it seems quite unlikely that these structures are found in it; it would seem most likely that the structure reflected by the circular dichroism and optical rotatory dispersion curves of phosvitin at pH 6.6 is an unordered conformation. A similar con-

⁵ Although it is common usage to refer to this conformation as random, we prefer the term "unordered"; in synthetic polypeptides, interactions between bulky side chains introduce considerable constraint upon the conformation and prevent the chain from behaving as a truly random coil; on the other hand, the exact conformation of each residue is independent of that of its neighbor's and is free to fluctuate within certain limits, rendering the over-all structure "unordered." Inside of a protein molecule, the definition of an unordered structure must include in addition lack of motion and repeatability from 1 molecule to another (15).

⁶ W. F. Harrington and N. V. Rao, in preparation.

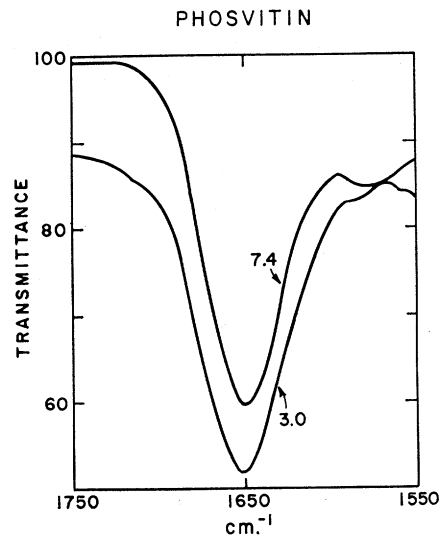


Fig. 3. Infrared spectra in the amide I region of phosvitin at pD 3.0 and 7.4. The pD 3.0 spectrum is displaced by 0.1 transmittance.

clusion has been reached by Jirgensons (19) from optical rotatory dispersion measurements.

The circular dichroism spectrum, obtained at pH 3.4, is shown in Fig. 2. It is characterized by deep negative absorption, maximal at 199 $m\mu$, with a broad shoulder at 220 $m\mu$ and small shoulders at 190 and 197 $m\mu$. In order to calculate the corresponding optical rotatory dispersion pattern, the circular dichroism spectrum was resolved in three ways: (a) two negative bands at 199 $m\mu$ and 222 $m\mu$, (b) a mixture of unordered and α -helical conformations with bands present at 196 (negative), 219 (positive), and 232.5 $m\mu$ (negative) for the unordered conformation (10, 11) and at 191 (positive), 206.5 (negative), and 221.5 (negative) $m\mu$ for the α helix (7-11, 20-23); (c) a mixture of unordered and antiparallel β structures with bands present

at 191 (positive) (11), 196 (positive) (8, 11, 14), and 217 (negative) (8, 11, 14, 24) $m\mu$ for the latter structure. The results of the Kronig-Kramers transformations for the three cases are shown on the right-hand side of Fig. 2 where they are compared with the experimental data (1). All three have a deep trough at 207 $m\mu$. Only the first two exhibit the rapid change of rotation between 207 and 200 $m\mu$ and the trough at 232 $m\mu$, found by Perlmann and Allerton (1) under the same conditions. The third case, containing a β conformation, does not have a trough at 232 $m\mu$, and thus must be eliminated as unlikely. Since the first case does not correspond to any known polypeptide conformation, it seems most likely that, at pH 3.4, phosvitin exists in unordered structure with approximately 10 to 20% α helix.

The infrared spectra in the amide I region, measured at pD 3.0 and 7.4, are shown in Fig. 3. Quite unexpectedly, they are almost identical and exhibit a maximum at 1650 cm^{-1} . Although this band position is consistent with that of an α helix in proteins (25–27),⁷ it appears too high for an unordered polypeptide or protein in D_2O .⁷ It may be possible that the strong electrostatic repulsion between the charged side chains of the protein imposes additional constraints on the modes of vibration of the peptide linkages and causes a displacement in the positions of the corresponding infrared absorption bands. In any case, the 1650 cm^{-1} band is highly inconsistent with the presence of β structure in any considerable amount.

The above examination of the circular dichroism and infrared spectra of phosvitin, together with the viscosity results of Jirgensons (3), suggests that the changes in optical rotatory dispersion, as the pH is shifted from 6.6 to 3.4, are probably the result of a transition from an extended unoriented chain, expected at high net charge, to a more compact structure which may contain up to 20% α helix.

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